# Aneuploidy in cancer

Trends in Cancer

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Opinion

# Aneuploidy in Cancer: Lessons from Acute Lymphoblastic Leukemia

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**Aneuploidy, the gain or loss of chromosomes in a cell, is a hallmark of cancer. Although our understanding of the contribution of aneuploidy to cancer initiation and progression is incomplete, significant progress has been made in uncovering the cellular consequences of aneuploidy and how aneuploid cancer cells self-adapt to promote tumorigenesis. Aneuploidy is physiologically associated with significant cellular stress but, paradoxically, it favors tumor progression. Although more common in solid tumors, different forms of aneuploidy represent the initiating oncogenic lesion in patients with B cell acute lymphoblastic leukemia (B-ALL), making B-ALL an excellent model for studying the role of aneuploidy in tumorigenesis. We review the molecular mechanisms underlying aneuploidy and discuss its contributions to B-ALL initiation and progression.**

## Highlights

Although aneuploidy is a hallmark of cancer, our understanding of its contributions to cancer initiation and progression remains limited.

Aneuploidy is physiologically associated with drastic cellular stress, but paradoxically favors tumor progression. Significant progress has been achieved over recent years in deciphering how aneuploid cancer cells self-adapt to promote tumorigenesis.

Different forms of aneuploidy represent the initiating oncogenic lesion in ~35% of patients with B-cell acute lymphoblastic leukemia (B-ALL), especially in childhood B-ALL.

Hyperdiploid B-ALL cells display a typical aneuploid-stress signature in which mitotic and chromosomal segregation defects are associated with impairments of the condensin complex and the aurora B kinase/chromosomal passenger complex (CPC), leading to cohesion defects and mitotic slippage.

## Aneuploidy in Cancer

The gain or loss of whole chromosomes, termed **aneuploidy** (see Glossary), was identified as a distinct feature of cancer cells more than a century ago by the German zoologist Theodor Boveri [1], and is now recognized as a major genomic insult in human cancers [2,3]. Aneuploidy is observed more frequently than any other oncogenic or tumor-suppressor mutation, and is found in ~90% of solid tumors and ~60% of hematological malignancies [4]. High-resolution, genome-wide analysis has revealed that ~25% of the genome in each cancer cell is affected by either chromosome-arm or whole-chromosome copy-number alterations [5]. Importantly, although recent studies have regarded both chromosome-arm gains and losses as aneuploidy, these are generated by different mechanisms. Thus, according to the classical definition of aneuploidy, we hereafter use the term 'aneuploidy' to refer to whole-chromosome gains and losses.

Despite its high incidence, our understanding of the contribution of aneuploidy to cancer initiation and progression is limited for several reasons. First, large chromosomal changes lead to alterations in hundreds of genes and/or pathways, making it challenging to precisely identify those genes involved in tumorigenesis. Second, aneuploidy is often associated with **chromosome instability (CIN)** and/or **microsatellite instability (MIN)** [6,7] that generate heterogeneous aneuploid karyotypes, making it difficult to distinguish the alterations that drive cancer growth from the numerous, apparently random, genetic alterations that occur during tumorigenesis. Third, aneuploidy is highly dependent on the cellular context and can both promote and inhibit tumor development in a tissue-specific manner [2]. Indeed, specific chromosome gains and losses have been shown to be linked to specific cell types and tumors [8,9]. Finally, the generation of models with specific aneuploidies remains technically challenging despite recent advances in microcell-mediated cell transfer [10] and in Cre/loxP [11] and CRISPR/Cas9 technology [12].

Given the widespread aneuploidy found in cancer, there has been long-standing debate on whether it is the cause or consequence of cancer. Because cancer cells are often defective in

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pathways associated with cell-cycle control and genome stability, one possibility is that aneuploidy arises as a consequence of disruption of these pathways [13,14]. Another possibility is that aneuploidy *per se* acts as a tumor-initiating event. In this line, it was shown that introduction of specific chromosomes into cells induces replication stress and further genomic instability [15], which may trigger cell transformation [11,16–18]. Of note, aneuploidy has been observed in some preneoplastic conditions such as Barrett's esophagus [19]. In addition, Down syndrome (DS) individuals harboring constitutional trisomy of chromosome 21 have a 200-fold higher risk of developing hematological malignancies [20]. Aneuploidy may clearly exert a tumor-promoting role in some cancers.

We review here the cellular and molecular mechanisms underlying aneuploidy, and discuss recent findings highlighting how aneuploidy contributes to the origin and progression of B-cell acute lymphoblastic leukemia (B-ALL). B-ALL represents an excellent model for studying the role of aneuploidy in tumor development because frequent chromosome gains and losses are observed as the sole genomic abnormalities in aneuploid B-ALL.

## Aneuploidy Is Caused by Chromosome Segregation Defects

The karyotype of most aneuploid tumors is within the range of diploid cells (46 chromosomes [4]), suggesting that chromosome imbalances occur largely through sequential losses and gains of chromosomes through CIN from an initial diploid state or by chromosome losses after endoreduplication of genetic material. Mechanisms of CIN include those that reduce **mitotic** fidelity leading to chromosome segregation defects [21].

### Improper Kinetochore–Microtubule Attachments

During prometaphase, nuclear envelope breakdown allows chromosomes to attach to microtubule-based 'spindles' via **kinetochores** (Figure 1). During metaphase, chromosomes are concentrated in a region at the mid-plane of the bipolar mitotic spindle known as the metaphase plate. The back-to-back geometry of sister kinetochores on each chromosome favors the attachment of each chromatid to microtubules arising from opposite poles (amphitelic attachments), thus allowing proper chromosome bi-orientation and segregation [22]. However, the stochastic nature of kinetochore–microtubule attachments frequently leads to chromosome misattachments to the spindle [23], such as syntelic, monotelic, and merotelic attachments (Figure 1). These attachment defects occur naturally in early **mitosis** and, in healthy cells, they are detected and corrected before anaphase onset by a system that includes different tension-sensing factors, including aurora B kinase and Mps1, to ensure faithful chromosome segregation [24]. Merotelic attachments are the most common cause of chromosome segregation errors because they are less likely to be detected by these factors and are often not corrected in cancer cells [21,25]. The persistence of merotelic attachments in human cancer cells is mediated by two nonexclusive mechanisms – a decreased rate of error correction and an increased rate of merotely. Notably, some cancer cell lines display hyperstable merotelic kinetochore–microtubule attachments in early mitosis [25], suggestive of inherent defects in correcting erroneous kinetochore–microtubule attachments. Accordingly, defects in several centromere/kinetochore proteins have been observed to reduce chromosome segregation efficiency by increasing the frequency of merotelic attachments and lagging chromosomes in anaphase [26,27]. However, mutations of kinetochore proteins in cancer are rare, presumably because these proteins are essential for life. However, imbalances in the levels of these proteins might act dominantly [23]. Correspondingly, other mechanisms such as epigenetic abnormalities in centrochromatin, such as loss of histone H3 lysine 4 dimethylation, that is typically found at the core centromere interspersed with the centromere-specific histone H3 CENP-A, were shown to lead to abnormal kinetochore protein levels resulting in chromosome mis-segregation [28,29].

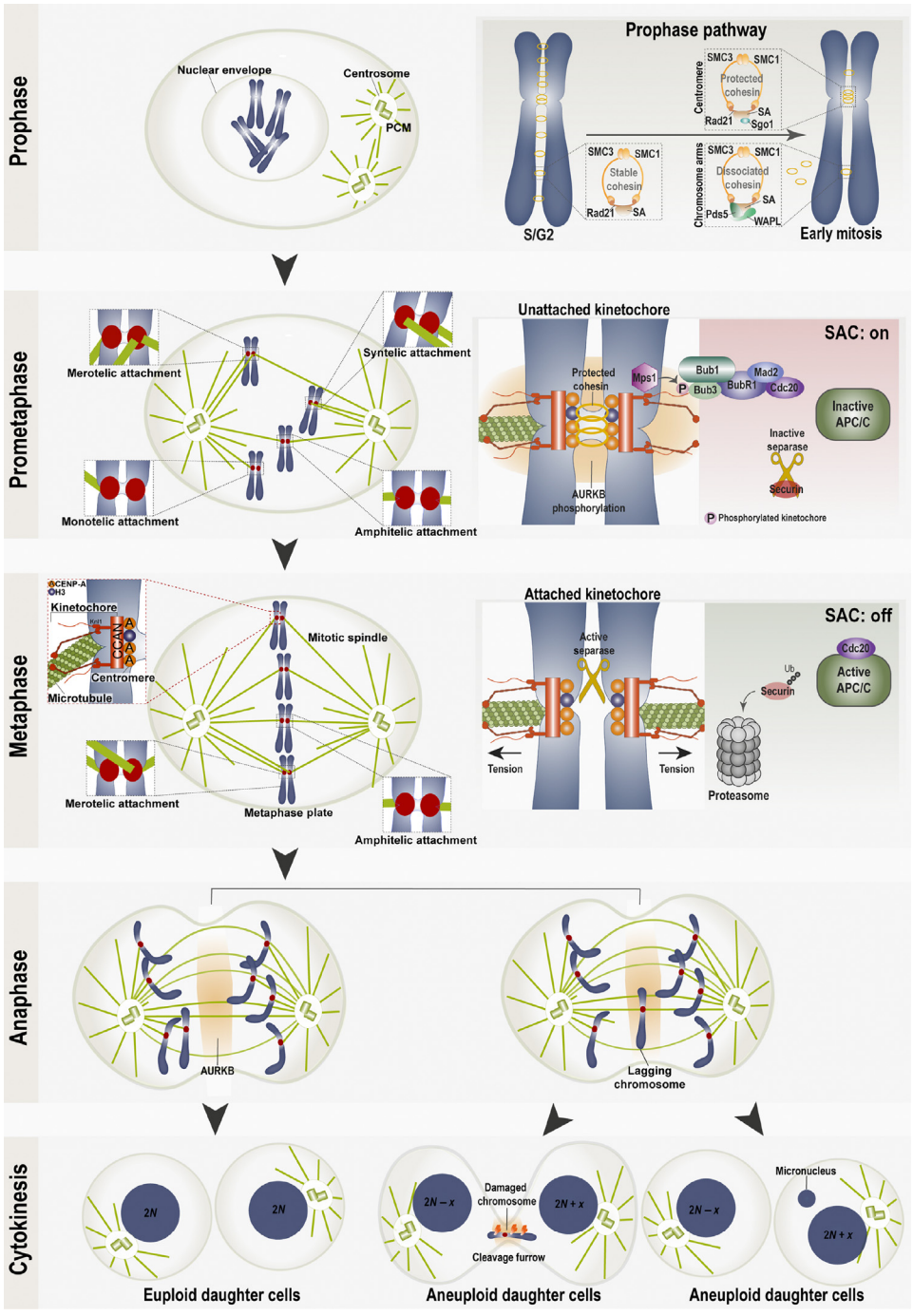


Figure 1. Mechanisms of Chromosome Mis-Segregation. Newly replicated chromatids are held together by cohesin, a multiprotein complex that in humans is composed of four subunits: two structural maintenance of chromosomes (SMC) ATPases (SMC1 and SMC3), the kleisin sister-chromatid cohesion protein 1 (RAD21), and stromal antigen (SA). During prophase, the bulk of cohesin is removed from chromosome arms by Pds5–WAPL and only remains at centromeres that are protected by shugoshin 1 (Sgo1). In prometaphase, kinetochore–microtubule attachments are initiated, including amphitelic attachments (sister kinetochores are attached to microtubules from opposite poles), syntelic attachments (both

*(Figure legend continued at the bottom of the next page.)*

## Glossary

**Aneuploidy:** gain or loss of one or more chromosomes resulting in an unbalanced chromosome number that is not an exact multiple of the haploid complement.

**Bridge-fusion-breakage cycle:** a mechanism of chromosome instability in which broken ends from different chromatids fuse, leading to the formation of a dicentric chromosome. This chromosome bridge breaks apart in mitosis as centromeres are pulled in opposite directions, generating broken ends that can reinitiate the cycle.

**Centrosomes:** microtubule organizing centers in metazoans; they consist of a pair of cylindrical centrioles surrounded by a matrix of proteins called the pericentriolar material, where most centrosome-associated proteins localize.

**Chromosomal passenger complex (CPC):** a heterotetrameric protein complex consisting of an enzymatic component aurora B kinase together with three regulatory components: INCENP (inner centromere protein), survivin, and borealin. The CPC dynamically localizes to different subcellular localizations throughout mitosis to regulate key events such as kinetochore–microtubule attachments, activation of the SAC, and assembly and maintenance of the outer kinetochore.

**Chromosome instability (CIN):** persistent gains and losses of chromosomes during cell division. It is important to underscore the difference between aneuploidy (the 'state' of the karyotype) and CIN (the 'rate' of karyotypic changes).

**Chromothripsis:** a mutational process by which up to thousands of clustered chromosomal rearrangements occur following the formation of micronuclei containing mis-segregated chromosomes.

**Cohesin:** a multiprotein ring-shaped complex that maintains replicated sister chromatids together from S-phase until anaphase onset.

**Euploidy:** a chromosome number that is an exact multiple of the haploid complement (*n*). It includes diploidy, triploidy, tetraploidy, and polyploidy. Ploidies other than diploidy decrease the fitness of cells but, compared with aneuploid cells, polyploid cells are relatively fit because their gene expression remains balanced and they therefore represent a more stable state than any aneuploidy.

### Defects in Chromosome Cohesion

In eukaryotes, newly duplicated sister chromatids are held together by **cohesin** [30]. Cohesin is also important to ensure the back-to-back orientation of kinetochores that facilitate chromosome amphitely (Figure 1), and is involved in other fundamental cellular processes such as DNA repair and transcription regulation [31]. Cohesin is removed from chromosomes in two stages during cell division to allow physical separation of the sister chromatids and accurate transfer of the genetic material into the two daughter cells. The bulk of cohesin is removed from the chromosome arms during early mitosis in a process driven by the cohesin-associated factor Pds5 and the cohesin-release factor WAPL that form the Pds5–WAPL complex (Figure 1). Residual cohesin is concentrated at centromeres where it is protected from removal by shugoshin-1 (from the Japanese 'guardian spirit'), resulting in the classical X-shaped mitotic chromosome [32] (Figure 1). Once all chromosomes have aligned at the metaphase plate, the protease separase removes centromeric cohesion and triggers anaphase onset, allowing separation of the sister chromatids to opposite spindle poles (Figure 1) [30,33]. Defective sister chromatid cohesion can result in chromosome mis-segregation and aneuploidy, and both mutations and abnormal expression of cohesin-related genes have been identified in different human cancers [34]. Given the essential role of cohesin in genome stability and transcription regulation, cohesion dysfunction might affect tumorigenesis in different ways; further studies will be necessary to shed light on the functional importance of mutations in cohesin and its interacting partners.

### Defective Spindle-Assembly Checkpoint

Eukaryotic cells have developed an evolutionarily conserved surveillance mechanism to prevent chromosome mis-segregation as a result of erroneous kinetochore–microtubule attachments, termed the **spindle-assembly checkpoint (SAC)**. The SAC pathway serves to restrain the protease separase, which cleaves the cohesin complex in anaphase (Figure 1). Impairment of the SAC allows precocious anaphase onset, and consequently significantly increases the frequency of chromosome mis-segregation [35]. Complete SAC ablation leads to massive chromosome mis-segregation and catastrophic cell death in cell lines [35,36] and is embryonic lethal in mice [37], but not in *Drosophila*, where chromosome attachment to the mitotic spindle occurs extremely rapidly [38]. By contrast, heterozygous mutations in different SAC proteins induce aneuploidy and tumorigenesis in mice [39–41]. Of note, mutations in *BUB1B*, that encodes the SAC protein BubR1, cause mosaic variegated aneuploidy in humans – a rare and recessive syndrome that is associated with mosaic aneuploidy and predisposition to cancer development [41].

### Supernumerary Centrosomes

**Centrosomes** are responsible for the formation of the mitotic spindle that is needed for proper chromosome segregation [42]. Coordination of the duplication and segregation cycles of centrosomes is essential to prevent chromosome segregation defects, because the number of centrosomes in a cell determines the number of spindle poles [43]. Duplication of the centrosome that is present in a G1 cell begins during S phase. In early mitosis the duplicated centrosomes nucleate

**Kinetochore:** a large protein complex that assembles at centromeres and binds to microtubules and directs chromosome segregation [90].

**Li–Fraumeni syndrome:** a rare autosomic dominant disorder caused by mutations in the *TP53* gene; the syndrome is associated with an increased risk of developing different types of cancer.

**Microsatellite instability (MIN):** clonal changes in the number of repeated DNA nucleotide units in microsatellite sequences. It arises in some tumors owing to inactivation of mismatch repair genes.

**Mitosis:** a highly orchestrated cellular process for faithful segregation of replicated chromosomes into two identical daughter cells.

**Spindle-assembly checkpoint (SAC):** an evolutionarily conserved surveillance mechanism that detects the presence of misattached kinetochores in mitosis and pauses division until all pairs of sister chromatids achieve the bioriented geometry.

sister kinetochores are attached to microtubules from the same pole), monotelic attachments (only one sister kinetochore is attached to the spindle), and merotelic attachments (both sister kinetochores are simultaneously attached to microtubules emanating from the same pole). Erroneous attachments are sensed by aurora B (AURKB) and Mps1 kinases that retain the mitotic checkpoint complex (Bub proteins, Mad2, and Cdc20) at unattached kinetochores and keep the spindle assembly checkpoint (SAC) activated, blocking the activity of the anaphase-promoting complex/cyclosome (APC/C). In metaphase, the tension established by amphitelic attachments causes the chromosomes to align at the metaphase plate. Correct kinetochore–microtubule attachments inactivate the SAC and allow APC/C activation, releasing separase to cleave cohesin rings at centromeres and promoting anaphase onset. Merotelic attachments are usually not sensed by aurora B, and these chromosomes frequently lag behind in anaphase, giving rise to either **euploid** or aneuploid daughter cells. Lagging chromosomes can be trapped in the cleavage furrow in cytokinesis, leading to chromosome breakage, or they segregate intact to a daughter cell where they can eventually encapsulate as a micronucleus. Abbreviations: PCM, pericentriolar material; Ub, ubiquitin.

the spindle microtubules and, later on in mitosis, the centrosomes from opposite spindle poles segregate to the daughter cells together with a complete set of chromosomes. An abnormal number of centrosomes can arise from defects in the centrosome replication cycle, segregation failure during mitosis, or cell fusion, leading to genome doubling and polyploidy. Remarkably, several observations support the hypothesis that polyploid cells represent early steps in tumor formation because centrosome duplication represents a source of merotelic attachments leading to chromosome mis-segregation [44–46].

## Consequences of Aneuploidy and the 'Aneuploidy Paradox' in Cancer

The deleterious effects of aneuploidy in physiology are well established from yeast to mammalian cells [16,47]. Aneuploidy is often lethal in multicellular organisms, and the few aneuploidies that do not cause lethality can reduce growth and trigger developmental abnormalities, leading to a substantial fitness cost under most circumstances [2]. Indeed, aneuploid mice generated by chromosome transfer die *in utero* at early stages [16]. Aneuploid cells share a set of phenotypes that are collectively known as the 'aneuploidy-stress phenotype' [48], which includes specific cellular responses such as lower proliferation rates, genetic and metabolic stress, and organismal effects including immune system activation (Box 1).

Despite the detrimental consequences of aneuploidy for cell fitness, the fact that it is a strikingly common feature in cancer creates a paradox in terms of its contribution to tumorigenesis, the so-called 'aneuploidy paradox' [2]. It is now clear that the effects of aneuploidy on tumorigenesis are much more complex than was initially proposed. Aneuploidy and CIN can act both as tumor-suppressors and tumor-initiators depending on the context [2,18]. A pan-cancer genomic analysis based on the potency and distribution of oncogenes and tumor-suppressor genes suggested that cumulative haploinsufficiencies and triplosensitivities drive aneuploidy patterns

### Box 1. Aneuploidy-Stress Phenotype

#### Cellular Effects of Aneuploidy

The most evident effect of aneuploidy is a reduction in cell proliferation and delayed cell-cycle progression at the G1–S transition [16,47,71]. Of note, the proliferation defects are not observed in aneuploid mouse embryonic stem cells or human pluripotent stem cells [17,72,73], suggesting a variable tolerance to aneuploidy depending on the developmental stage of the cell. Lower proliferation rates are most likely a response to other cellular stresses associated with aneuploidy, such as gene expression and proteotoxic stresses. In addition, aneuploid cells have a characteristic gene expression signature in which gene expression networks are globally disturbed, similarly to the environmental stress response in yeast [47]. In mammalian cells, the aneuploidy-stress response involves the conserved downregulation of pathways involved in nucleic acid metabolism, including replication, DNA repair, transcription, and RNA processing, as well as upregulation of pathways associated with autophagy, lysosomal pathways, and membrane metabolism, metabolic pathways such as glycolysis, and pathways associated with the inflammatory response [71]. The increased gene expression triggers proteotoxic stress as cells attempt to cope with the unbalanced excess of proteins generated by additional chromosome numbers. Proteotoxic stress is characterized by impaired protein folding, activation of degradation pathways, and accumulation of cytoplasmic protein aggregates with consequent accumulation of autophagosomes [74,75]. These pathways allow aneuploid cells to maintain protein homeostasis against a background of elevated gene expression. In addition to metabolic stresses, aneuploidy generates further genomic instability, leading to cancer genome evolution and adaptation. Genomic instability in aneuploid cells is triggered by different mechanisms such as replication stress [15], chromosome **bridge-fusion-breakage cycles** [76,77], and **chromothripsis** [78].

#### Organismal/Physiological Effects

Aneuploidy and CIN activate the innate immune response through the cGAS–STING pathway (cyclic GMP-AMP synthase–stimulator of interferon genes), a cellular defense against viral infection [79,80]. The cGAS–STING pathway detects DNA double-strand breaks (DSBs) that leak from micronuclei into the cytoplasm and induce the transcriptional activation of inflammatory pathways, such as type I interferon signaling and the senescence-associated secretory phenotype (SASP), thus providing a link between chromosome mis-segregation, aneuploidy, and innate immune signaling [80]. Indeed, the type I interferon pathway is consistently upregulated in aneuploid mammalian cells, and the expression levels of proinflammatory cytokines increase upon chromosome mis-segregation [79,80]. Thus, aneuploidy triggers signals for cell self-elimination that may serve as a means for cancer cell clearance.

and shape the cancer genome in human primary tumors [49]. These genetic effects are tissue-specific [9], and this may explain the tumor type-specificity of the observed patterns of chromosome gains and losses. Collectively, these data highlight the balance between the deleterious and beneficial consequences of aneuploidy and CIN during the early steps of tumor evolution.

## Aneuploidy in Hematological Neoplasms

Dramatically different biases of individual chromosome gains and losses have been reported between solid tumors and hematological neoplasms, and these could reflect their fundamentally different etiologies [4]. Whereas solid tumors preferentially lose chromosomes, this relationship is not so clear in hematological neoplasms, which show a more balanced rate of chromosome gains and losses [4]. Notably, the most frequently gained chromosome in hematological neoplasms is chromosome 21, which has a clear bias towards loss in solid tumors. Consistently, patients with DS, with constitutional trisomy 21, have a >200-fold increased susceptibility to hematological neoplasms, including acute megakaryocyte-erythroid leukemia and B-ALL, but appear to be protected from solid tumors [50]. Importantly, trisomy 21 and its mouse homolog (trisomy 16) have been shown to perturb the development of hematopoietic stem and progenitor cells (HSPCs), specifically B lymphoid differentiation [20,51,52]. These data suggest that trisomy 21 influences cell-autonomous differentiation and transformation phenotypes in progenitor B cells, thus linking specific aneuploidies to the development of hematological neoplasms.

Because hematological neoplasms are characterized by the presence of recurrent chromosomal abnormalities, cytogenetic studies at diagnosis remain essential to (i) confirm clonality, (ii) classify patients based on World Health Organization (WHO)-defined subgroups, and (iii) stratify patients according to their prognosis to determine the most suitable treatment (revised international prognostic scoring system, IPSS-R) [53]. Beyond structural chromosome abnormalities, aneuploidy is frequent in some hematological neoplasms and is associated with specific clinical outcomes (Table 1). Although it is generally challenging to define which chromosomal abnormality represents the primary change, chromosome gains and losses are mostly accompanied by additional cytogenetic aberrations that reflect disease progression in most hematological neoplasias. Nevertheless, aneuploidy represents the primary event in some hematological neoplasms (Table 1), where ALL

Table 1. Main Aneuploidy Cytogenetic Abnormalities in Hematological Neoplasms[a]

| Neoplasm | Cytogenetic alterations | Clinical outcome | Refs |
|---|---|---|---|
| Chronic myelomonocytic leukemia (CMML) | –Y | Favorable | [81] |
| | +8, CK | Poor | |
| Myelodysplastic syndromes (MDS) | –Y | Very favorable | [53] |
| | –7[b] | Poor | |
| | CK | Very poor | |
| Acute myeloblastic leukemia (AML) | –5[b], –7, +8, –17 or abn(17p), CK, MK | Poor | [82] |
| Chronic lymphocytic leukemia (CLL) | +12[b], +18 | NPA | [83] |
| Multiple myeloma (MM) | +1, +3, +7, +11, +15 | NPA | [84,85] |
| Non-Hodgkin lymphoma (NHL) | +3, +5, +7, +12, +18 | NPA | [86] |
| Myeloproliferative neoplasms (MPN) | -7[b], +8[b], +9[b], –Y[b] | NPA | [53] |
| Acute lymphoblastic leukemia (ALL) | +21, hyperdiploidy (>50 chr)[b] | Favorable | [87,88] |
| | Hypodiploidy (<40 chr)[b] | Poor | |

[a]Abbreviations: abn, abnormality; chr, chromosomes; CK, complex karyotype; del, deletion; MK, monosomal karyotype; NPA, no prognosis associated.
[b]As a single abnormality.

is the best example, where highly aneuploid karyotypes are observed without other detectable cytogenetic abnormalities (Box 2). The fact that chromosome gains and losses represent the unique cytogenetic abnormality in most aneuploid B-ALL cases makes this an excellent model for studying the pathogenic effects of aneuploidy on the initiation and progression of hematological cancers.

## Etiology of Aneuploidy in Childhood B-ALL

Genomic studies mapping the temporal relationship between chromosomal gains or losses and specific mutations in aneuploid chromosomes of B-ALL have revealed that aneuploidies arising *in utero* are the initiating event in both hyperdiploid and hypodiploid childhood B-ALL [54,55]. These findings are consistent with studies showing that hyperdiploidy is present at birth, as shown by genetic analysis of monozygotic twins with concordant and discordant high hyperdiploid (HeH) B-ALL [56], the presence of hyperdiploid cells in cord blood samples [57], and the identification of clonotypic *IGH* rearrangements in Guthrie cards (neonatal blood tests that are also known as 'heel-prick tests') from patients who later developed HeH-B-ALL [58]. Overall, these data strongly suggest that aneuploidies are early initiating events acquired prenatally during fetal hematopoiesis, as has been observed for other common reciprocal translocations in B-ALL, and also that a secondary postnatal mutational event is necessary to promote leukemia development [59,60]. This requirement for secondary cooperating oncogenic insults is suggested by the variable postnatal latency period of the disease and by the inability of common B-ALL fusion genes to promote leukemia in either transgenic mice *per se* [61] or human HSPCs [62]. Accordingly, aneuploid B-ALL cases typically have recurrent secondary mutations or copy-number alterations involving genes such as *CDKN2A*, *PAX5*, *IKZ1*, and *ETV6*, as well as mitogen-activated protein kinase (MAPK) signaling pathway genes (*RAS*, *FLT3*, and *PTPN11*) and histone modifiers [63,64].

The cellular and molecular mechanisms leading to aneuploidy in B cell progenitors are largely unknown because the aberrant mitosis that generates the gross chromosome gains and losses

### Box 2. Aneuploidies in B Cell ALL

Aneuploidies are common cytogenetic abnormalities in B-ALL, particularly in pediatric cases that account for ~30% of B-ALL cases. The most frequent aneuploidies are hyperdiploid karyotypes, generally with several chromosome gains, and these represent the largest cytogenetic subgroup in childhood B-ALL (25–30% of cases) [67]. Hyperdiploid B-ALL is classified according to the chromosome gains into low-hyperdiploid (HeL-B-ALL), with 47–50 chromosomes; high-hyperdiploid (HeH-B-ALL), with 51–67 chromosomes; and near-triploid/tetraploid, with >67 chromosomes (Figure IA–C). Among hyperdiploid B-ALL cases, the most common group is HeH-B-ALL, which is associated with a favorable prognosis and has a long-term overall survival (OS) of >90% [67]. Chromosome gains in B-ALL are not random, and HeH-B-ALL shows preferential gains of chromosomes 4, 6, 10, 14, 17, 18, 21, and X. Further research will be necessary to establish the individual contributions of each chromosome gain in both leukemia origin/initiation and (sub)clonal intratumor heterogeneity.

Although HeH-B-ALL is the most common aneuploidy in childhood B-ALL, chromosome losses are also observed and are classified as hypodiploid B-ALL, which represents <10% of patients with B-ALL [89]. Based on the number of chromosome losses, hypodiploid B-ALL cases are classified as high-hypodiploid (HoH-B-ALL), with 40–45 chromosomes; low-hypodiploid (HoL-B-ALL), with 30–40 chromosomes (Figure ID); and near-haploid (Figure IE), with <30 chromosomes. Most hypodiploid cases are HoH-B-ALL, but this group is genetically heterogeneous and does not have a clear prognostic value. By contrast, HoL-B-ALL and near-haploid B-ALL, that account for ~2% of pediatric B-ALL cases, have an important prognostic value but an extremely dismal prognosis (5 year OS of <25%) [89]. Similarly to hyperdiploid B-ALL, cases of hypodiploid B-ALL display a preferential loss of chromosomes. In near-haploid B-ALL, retained disomies principally comprise chromosomes X/Y, 8, 10, 14, 18, and 21, whereas HoL-B-ALL shows preferential retention of disomies X/Y, 1, 5, 6, 8, 10, 11, 14, 18, 19, 21, and 22. Half of all patients with HoL-ALL frequently harbor 'inherited' *TP53* mutations, suggesting that *TP53*-mutated HoL-B-ALL is a clinical evolution of **Li–Fraumeni syndrome** [64]. Notably, half of all hypodiploid B-ALL cases show chromosomal doubling in the hypodiploid clone, resulting in clones with 50–78 chromosomes (Figure ID). The doubled clones frequently represent the major leukemic clone at diagnosis, leading to 'masked-hypodiploidy' [89], which is clinically challenging because these patients could be erroneously classified and be treated as hyperdiploid B-ALL while being at high risk of treatment failure.

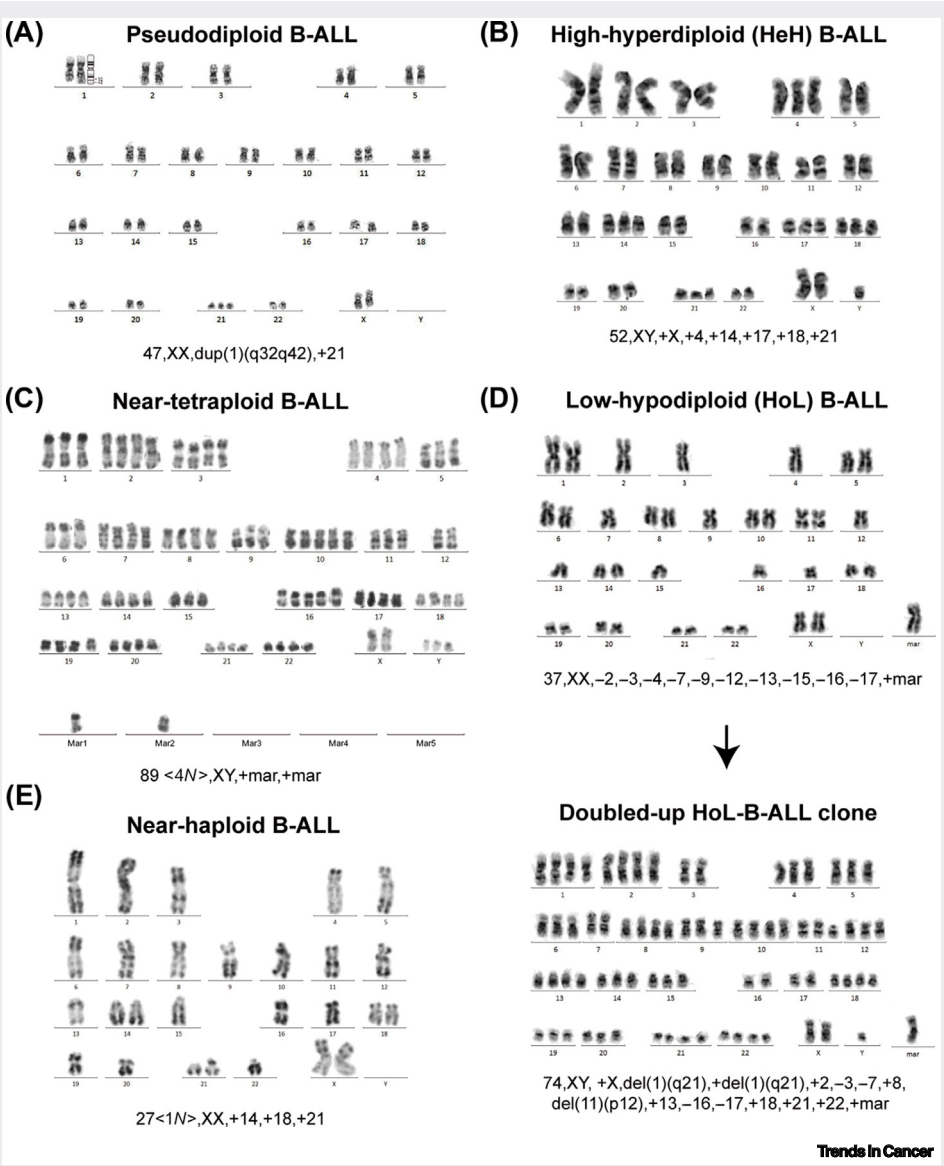


Figure I. Aneuploid Karyotype Configurations Found in B-ALL. (A) G-banded karyotype of pseudodiploid B-ALL blasts, where the only aneuploidy observed is +21. (B) G-banded karyotype of high-hyperdiploid B-ALL blasts. (C) R-banded karyotype of near-tetraploid B-ALL blasts. (D) G-banded karyotype of low-hypodiploid B-ALL blasts (above) and a doubled-up clone observed in the same patient sample with 74 chromosomes (below). Both clones were seen at disease presentation. (E) R-banded karyotype of near-haploid B-ALL blasts. Abbreviation: B-ALL, B cell acute lymphoblastic leukemia; mar, structurally abnormal marker chromosome in which no part can be identified.

in aneuploid B-ALL cannot be directly observed. The evidence for the cellular mechanisms leading to aneuploid B-ALL is thus purely circumstantial. Studies of microsatellite sequence allelic ratios in tetrasomic chromosomes and uniparental disomies (UPDs) in HeH-B-ALL cases strongly suggest that hyperdiploid karyotypes arise as a result of simultaneous chromosome gains in a single abnormal mitosis in most cases (~70%), or by chromosome losses from a tetraploid intermediate (~30%) [65]. However, the cellular mechanisms leading to these mitotic defects remain unknown.

Whole-genome sequencing analyses in large cohorts of patients with aneuploid B-ALL have not identified recurrent mutations or defects in genes associated with mitosis or cell division that could explain the causative mechanisms for these aneuploidies in B cell progenitors [63,64]. However, no studies on the mitotic mechanisms involved in aneuploid B-ALL have been performed. We recently reported the first and most comprehensive analysis of mitotic progression of HeH-B-ALL using primary blasts and primograft-derived xenograft models [66]. The studies revealed that hyperdiploid B-ALL blasts show a delay in early mitosis in prometaphase as a result of defects in chromosome alignment that ultimately lead to chromosome segregation errors, as observed by the presence of a higher frequency of anaphase bridges and lagging chromosomes in late mitosis compared with non-hyperdiploid B-ALL samples. Accordingly, we observed karyotype variability in HeH-B-ALL samples with a major clone and a series of smaller clones, suggesting a mild CIN phenotype. Although the presence of CIN in HeH-B-ALL remains controversial [67], the analysis is supported by the direct visualization of chromosome mis-segregation in mitosis, and favors the presence of CIN observed by single-cell analyses such as interphase-FISH (fluorescence *in situ* hybridization) in HeH-B-ALL samples [68,69]. Biochemical analysis of dividing B-ALL blasts revealed that the condensin complex is functionally impaired in HeH-B-ALL blasts, leading to chromosome hypocondensation, loss of centromere stiffness, and mislocalization of the **chromosomal passenger complex (CPC)** that contains aurora B kinase as its enzymatic core. Chromosome architecture defects have recently been reported by other authors using Hi-C (high-throughput sequencing and chromatin conformation capture) analysis of primary HeH-B-ALL samples [70] and can explain very well the poor chromosome morphology that clinical cytogeneticists historically encountered in metaphase spreads of HeH-B-ALL. HeH-B-ALL blasts showed chromatid cohesion defects and loss of SAC efficiency, leading to mitotic slippage, perhaps because aurora B kinase fails to localize to the inner centromere to protect chromatid cohesion and allow proper SAC activity; this provides a direct link between chromosome misalignment and segregation defects in late mitosis. Overall, these data constitute direct evidence for mitotic and chromosome defects in HeH-B-ALL cells and highlight the molecular mechanisms involved in the pathogenesis of this frequent subgroup of childhood B-ALL. Whether these mechanisms are involved in the origins of hyperdiploidy or are the consequence of chromosome gains in B cell progenitors remains an open question that requires further investigation.

## Concluding Remarks

An important caveat to these studies is the precise identification of causative versus consequential defects with a view to establishing the mechanism responsible for the different aneuploidies in B-ALL. In this sense, mouse models can provide important insights into the mechanisms underlying mitotic defects in B cell progenitors and the effects of aneuploidy on leukemia initiation and progression. A better understanding of the biology of hyperdiploid and hypodiploid B-ALL subtypes will open up new avenues for *in vivo* modeling aneuploidy by genetically engineering HSPCs. This will be crucial for answering several key questions about the role of aneuploidy in leukemia initiation and progression (see Outstanding Questions). The new data obtained from these studies will translate into better diagnosis and treatment of patients with these common forms of leukemia.

## Outstanding Questions

Is there a common pathogenic mechanism that is shared between hyperdiploid and hypodipoid B-ALL?

What is the contribution of mitotic and chromosome segregation defects to the origin and progression of aneuploidy in B-ALL?

What are the precise cellular and molecular mechanisms that underlie disease initiation and progression in aneuploid subtypes of B-ALL?

What secondary alterations are necessary (if any) to develop leukemia in aneuploid B-ALL?

What is the contribution of each chromosome gain or loss in both leukemia initiation and progression? Are chromosome-number alterations stochastic or hierarchical?

Does intratumor heterogeneity contribute to progression of aneuploidy B-ALL?

Why is aneuploidy preferentially observed in pediatric B-ALL rather than in adult B-ALL or acute myeloid leukemia (AML)? Is there a developmental link between aneuploidy and B cell leukemia?

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